Talanta 136 (2015) 42–46

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Disposable immunosensor for human cytomegalovirus glycoprotein B detection

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ARTICLE INFO

Article history:
Received 30 August 2014
Received in revised form
12 December 2014
Accepted 16 December 2014
Available online 6 January 2015

Keywords:
Immunosensor
Screen-printed electrodes
Human cytomegalovirus
Glycoprotein B
Anodic stripping analysis

ABSTRACT

Combining the advantages of the biosensor field with the problem of detecting Human Cytomegalovirus (HCMV) in human samples, an inexpensive, simple and disposable electrochemical immunosensor for glycoprotein B detection in urine is proposed. Glycoprotein B has been chosen once is the dominant antigen of HCMV. The approach is based on a sandwich-type immunoassay, with the HCMV glycoprotein B sandwiched between the Anti-HCMV antibody adsorbed onto the working electrode, and the Anti-HCMV labeled with gold nanoparticles. Glycoprotein B detection was carried out through electrochemical stripping analysis of silver nanoparticles quantitatively deposited on the immunosensor through catalysis by the nanogold labels. The influence of different steps in the immunosensor construction, namely the silver deposition time, silver enhancer concentration, antibody concentration, BSA concentration and glycoprotein B incubation time, were examined and optimized. The method showed a linear dependence between glycoprotein B concentration and the corresponding anodic stripping peak current, resulting in detection limits of 3.3 ± 1.7 ng/mL for samples prepared in buffer and 3.2 ± 0.2 ng/mL for urine samples, suggesting that the biological matrix does not interfere with the immunosensor detection capability. Given its mode of preparation, by physical adsorption of the capture antibody in the working electrode, the immunosensor also exhibited an acceptable reproducibility, with a residual standard deviation of 13.5% for samples prepared in buffer and 11.2% for urine samples, thereby presenting a promising development potential for clinical applications.

1. Introduction

Human Cytomegalovirus (HCMV) is the most usual name for human herpesvirus 5 [1,2]; like all herpes viruses, HCMV undergoes latency and reactivation in the host cell, so once a person is infected, the virus is not eliminated from the body but persists to cause a low-grade chronic infection or remains in latent stage, allowing further transmission of the virus to new hosts [1,3]. Humans are the only known receptors for this virus and the transmission is facilitated by mucous contact. The reactivation of HCMV to a state of active replication with potential to induce disease and transmission to a new host can occur in a situation of cellular immunity suppression induced by a disease [2]. Thereby, in immunosuppressed individuals, as transplanted ones, persons infected by the Human immunodeficiency virus (HIV) and those with an immature immune system, like fetuses and newborns, the infection can be severe or even fatal [1,2,4]. All of the usually used methods for HCMV detection require a long period of time to perform or are costly which is problematic to use as point of care. Thus, there is a need to develop a method that is fast, effective and inexpensive for the diagnosis of this virus.

In recent years, electrochemical biosensors are widely used to determine various substances with different properties and for continuous monitoring of biological processes [5,6]. Bioanalytical assays such as immunoassays (IAs), which use specific antigen–antibody complexation, are also very important in many fields [7]. Moreover, IAs with electrochemical detection can offer enhanced sensitivities and reduced instrumentation costs compared to their counterparts using other transducing elements [5]. Warsinke et al. [8] already showed electrochemical IAs as promising alternatives to existing immunochemical tests for the development of hand-held devices which can be used for point of care measurements. Also, screen printed electrodes (SPE) contribute to develop

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http://dx.doi.org/10.1016/j.talanta.2014.12.016
0039-9140/

miniaturized, easy to handle, reliable, versatile and inexpensive IAs devices, which produce results within a few minutes [5].

Accurate detection of HCMV glycoprotein B (gB) in body fluids, like urine and saliva, where viral loads are higher, through immunoassay methods shows promising applications in screening and diagnosis of infection by HCMV. During HCMV acute infection, specific antibodies are generated against a large number of structural and non-structural proteins, but just glycoproteins B and H induce antibodies capable of neutralizing virus and eliminating infected cells. Furthermore, gB is the dominant antigen existing in the envelope of HCMV and approximately 100% of infected individuals develop antibodies against this protein. Thus, HCMV glycoprotein B can be viewed as a promising component to be used in the development of diagnostic tests for HCMV [2,10]. In this work, using the screen-printed technology, an electrochemical immunosensor is developed for human cytomegalovirus glycoprotein B detection.

Once antibodies are usually not electrochemically active within the desired potential range, redox-active compounds need to be applied as labels [8]. Silver deposition catalyzed by gold nanoparticles (AuNPs) has great interest in metalloimmunoassays, since this nanoparticles have well-known catalytic properties on silver ions reduction [11]. Thus, the use of a sandwich type immunoassay with a secondary antibody labeled with AuNPs allows the quantitative precipitation of silver nanoparticles (AgNPs), thereby improving the sensitivity of the immunosensor [12]. Subsequent quantification is made by dissolving the AgNPs and measuring its oxidation by anodic stripping voltammetry. The present work describes a sandwich type immunosensor for the detection of human cytomegalovirus glycoprotein B using the above-mentioned principle. To the best of our knowledge, this is the first time such a detection method is used in the quantification of this human cytomegalovirus envelope glycoprotein. Also, the developed biosensor proved to be effective in the detection of this glycoprotein, when present in human urine samples.

2. Material and methods

2.1. Reagents and apparatus

Cytomegalovirus glycoprotein B and Anti-Cytomegalovirus glycoprotein B antibody were purchased from Abcam, Cambridge, United Kingdom. Bovine serum albumin (BSA), chloroauric acid, Tris ((hydroxymethyl)aminomethane), nitric acid, potassium carbonate, tris–sodium citrate, potassium chloride, Tween-20 and silver enhancer solutions A and B were obtained from Sigma-Aldrich, Madrid, Spain. Urine samples were obtained from healthy volunteers who had given informed consent. All the reagents used were of analytical grade and Milli Q water (Millipore, Bedford, USA) was employed for preparing all solutions.

In the fabrication of screen-printed electrodes several inks were used, namely Electrodag PF-407 A (carbon ink), Electrodag 6037 SS (silver/silver chloride ink) and Electrodag 452 SS (dielectric ink) supplied by Achenson Colloiden (Scheemda, Netherlands). The SPEs, composed by carbon auxiliary and working electrodes and a Ag/AgCl pseudo-reference electrode, were produced on a DEK 248printing machine (DEK, Weymouth, United Kingdom), using polyester screens with appropriate stencil designs mounted at 45° to the printer stroke.

Electrochemical measurements were made using an Autolab PGSTAT128N electrochemical system with the General Purpose Electrochemical System (GPES) software version 4.9 (Echo Chemie, Utrecht, The Netherlands). The solution's pH was measured with a Hanna instrument HI 221 pH meter (USA).

2.2. Preparation of Au NP-labeled antibodies

For the preparation of colloidal AuNps, 100 mL of 0.01% $HAuCl_4$ solution was boiled with vigorous stirring, and 2.5 mL of 1% tris–sodium citrate solution was quickly added to the boiling solution. It is expected that the solution becomes red, indicating the formation of AuNps. After this, the solution was left stirring in order to cool down [13].

The Au-labeled antibody was obtained by adding 10 μL of a 1 mg/mL antibody solution to a cold colloidal gold suspension (1 mL) adjusted to pH 9.0, with 0.1 M K_2CO_3 solution. Next the solution was stirred at room temperature for 60 min. Then, the conjugate was centrifuged at 4800 rpm for 30 min, twice. Finally the soft sediment was suspended in 50 mM pH 7.2 Tris–HNO_3 solution (containing 1% BSA).The solution was stored at 4 °C [13,14].

2.3. Immunosensor preparation and measurement procedure

The immunosensor was constructed by adsorption immobilization of the corresponding capture antibodies on the screen-printed carbon working electrode surface. First, 3 μL of 1 μg/mL of anti-cytomegalovirus glycoprotein B capture antibody solution was deposited on the carbon working electrode surface and reacted for two hours at 4 °C in a 100% moisture saturated environment. Subsequently, the excess of antibodies were washed with washing buffer (1% Tween 20 in 50 mM pH 7.2 Tris–HNO_3 solution) and pH 7.2 Tris–HNO_3 solution. Next a drop of 3 μL of blocking solution (BSA 5 mg/mL in 50 mM pH 7.2 Tris–HNO_3 solution) was applied and incubated for 60 min at room temperature, which was followed by washing again with washing buffer and pH 7.2 Tris–HNO_3 solution. The blocking solution blocks the possible remaining active sites and avoids non-specific adsorption [9,12].

To carry out the immunoreaction and electrochemical measurement, the immunosensor was firstly incubated, with 3 μL of a cytomegalovirus glycoprotein B solution (different concentrations), for 20 min, followed by washing it with washing buffer and pH 7.2 Tris–HNO_3 solution. Then, it was incubated with 3 μL of AuNP-labeled anti-cytomegalovirus glycoprotein B solution for 20 min (under dark). After washing again with washing buffer and Tris–HNO_3 solution, 2 μL of silver deposition solution was delivered on the surface of working electrode and incubated for 4 min (under dark), followed by rinsing with water. Finally differential pulse voltammetry was performed from −0.06 V to 0.06 V in 1.0 M KCl solution to record the current.

3. Results and discussion

As mentioned, the present proposed approach is based on a sandwich-type immunoassay, with the HCMV glycoprotein B sandwiched between the Anti-HCMV antibody adsorbed onto the working electrode and the Anti-HCMV labeled with gold nanoparticles. In the presence of a silver deposition solution, the attached AuNP catalyzes the reduction reaction of silver ions, leading to the formation of AgNPs. Subsequently, the silver nanoparticles suffer an oxidation process, by means of anodic stripping, and the analyte is quantified.

To achieve better analytical performance several parameters were optimized, namely the effect of silver deposition time on stripping analysis, the silver enhancer concentration, the antibody and BSA concentration, and the glycoprotein B incubation time. Under optimal operation conditions, immunosensor performance was tested. Finally, in order to evaluate the feasibility of the proposed immunosensor, for real sample analysis, the device was used for the determination of human cytomegalovirus glycoprotein B in spiked urine human samples, by using the standard addition method.

3.1. Optimization of detection conditions

3.1.1. Effect of silver deposition time on stripping current

Results for the effect of silver deposition time on stripping current are displayed in Fig. 1. It can be seen that the stripping-current response increased greatly with the increasing silver deposition time until 4 min, after which it remained constant. Furthermore, after 6 min. of silver deposition, the electrochemical signal showed alterations (data not shown); thus, 4 min. was adopted as the optimal silver deposition time in this work.

3.1.2. Effect of silver enhancer concentration

When running blank experiments (bare electrode) the presence of a silver oxidation signal was always observed. In order to find the concentration where the gold nanoparticles have the ability to amplify the signal with respect to the blank, different dilutions of silver enhancer solutions were tried. The oxidation signals of silver nanoparticles onto the bare electrode surface and on the electrode modified with immobilized antibodies labeled with gold nanoparticles were compared. It was observed that a 20-fold dilution of silver enhancer is a very concentrate solution, and no difference was found in the signals intensity (Fig. 2a). The best results were reached in the presence of a 140-fold dilution of the silver enhancer solutions (A and B) (Fig. 2b); accordingly, this dilution was adopted as the optimal concentration for the silver enhancer.

3.1.3. Effect of antibody concentration

The antibodies are one of the most expensive components used in the immunosensor construction, so in order to decrease the device cost without compromising its performance, the effect of antibody amount used during immobilization was studied. Five different antibody concentrations were tested (0.02; 0.125; 0.25; 0.5 and 1 μg/mL) using a concentration of 20 ng/mL gB and a 140-fold diluted silver enhancer solution; the obtained results are shown in Fig. 3. With the exception of 0.02 μg/mL, which presents the lowest current intensity, at all the other concentrations the current intensity is kept constant at approximately 27 μA. Nevertheless, we have chosen the 0.25 μg/mL concentration as the optimal one as it presents less dispersion.

3.1.4. Effect of BSA concentration

In order to avoid non-specific binding and improve the analysis, the effect of BSA (blocking agent) concentration on stripping-current response was analyzed. The current intensity and signal dispersion of AgNPs for 20 ng/mL gB in 1.0 M KCl was studied in a range of BSA concentrations from 0% to 8%. The results (Fig. 4) exhibited a constant response from 2% to 6%, after which current decreases probably due to the blockage of antigen binding sites on the immobilized antibody. Although 2% seems to be the optimal BSA concentration, all of our experiments were performed using a concentration of 5%. Nevertheless, this cannot be considered an issue, since this concentration is integrated in the constant current response zone.

3.1.5. Effect of gB incubation time

The time used for the complexation reaction between antigen and antibody is an important step affecting the analytical performance of the immunosensor. This optimization can reduce the time needed to complete the assay. Incubations of 20 ng/mL of HCMV gB, during 0, 10, 20, 30, 40, 50 and 60 min, were carried out.

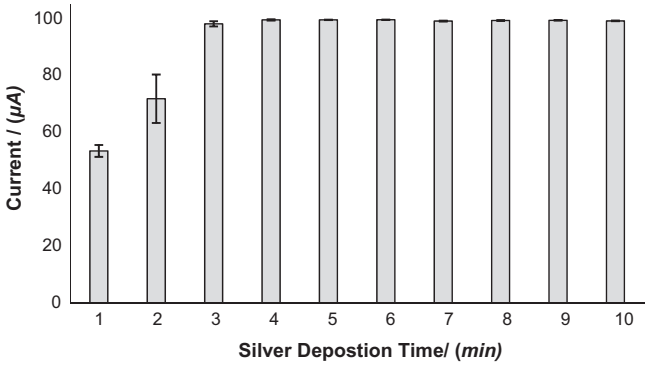

Fig. 1. Effect of silver deposition time on stripping-current response of AgNPs for 15 ng/mL Glycoprotein B in 1.0 M KCl. Results from independent triplicate tests, error bars represent the standard error.

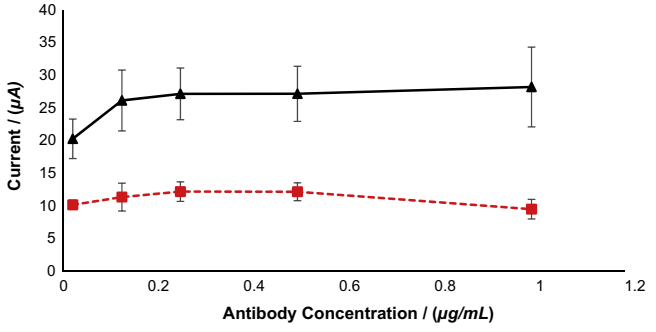

Fig. 3. Effect of antibody concentration on stripping-current response of AgNPs for 20 ng/mL Glycoprotein B in 1.0 M KCl. The mean of eight independent analyses is shown, and error bars indicate standard errors. Blank; Assay.

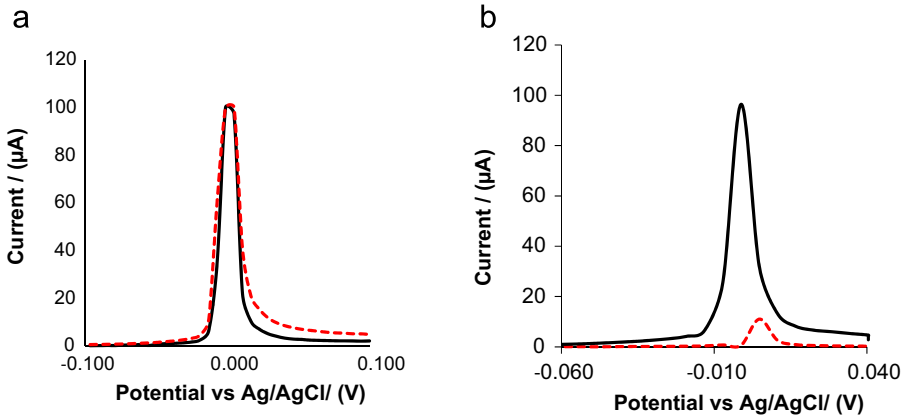

Fig. 2. Electrochemical signal intensity, for 15 ng/mL Glycoprotein B in 1.0 M KCl, at different concentrations of silver enhancer (study made in triplicate). (- - -) bare electrode; (—) electrode modified with AbAuNPS. (a) 20-fold dilution; (b) 140-fold dilution.

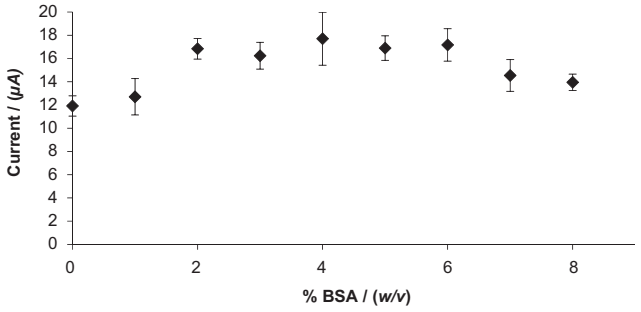

Fig. 4. Effect of BSA concentration on stripping-current response of AgNPs for 20 ng/mL Glycoprotein B in 1.0 M KCl. Each data point represents the average of eight tests and standard error is represented by the error bars.

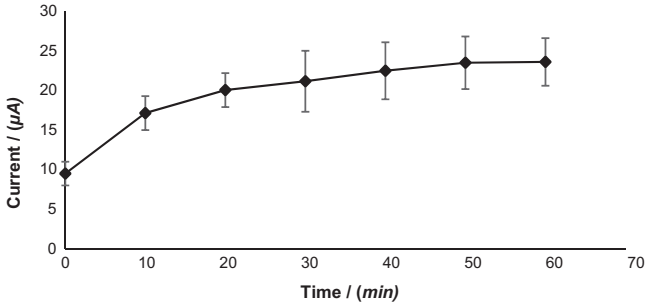

Fig. 5. Effect of antibody on stripping-current response of AgNPs for 20 ng/mL Glycoprotein B in 1.0 M KCl. The mean of eight independent analyses is shown, and error bars indicate standard errors.

The evaluation of the stripping voltammetric response (Fig. 5) indicated that after 20 min, current intensity is maintained constant, showing saturation in the formation of the sandwich immunocomplex [12]. Because 20 min presents the highest current intensity with the lowest standard error (Fig. 5), this incubation time was selected to run the immunoassay.

3.2. Immunosensor performance

With the presented sandwich-type immunoassay format, the quantitatively deposited AgNPs could be easily detected by anodic stripping voltammetric analysis in KCl solution. In Fig. 6a we can observe the change in stripping current as a function of glycoprotein B concentration. Current increases linearly with analyte concentration (Fig. 6b) in the range from 5 ng/mL to 15 ng/mL. Calibration curves (made in triplicate) (Table 1) showed good linear correlation coefficients between current intensity and gB concentration.

To test the biological matrix effect on the immunosensor, a 2-fold diluted urine sample (prepared in 50 mM Tris–HNO_3 buffer pH 7.2) from a healthy patient was used to obtain solutions with different gB concentrations. As observed with gB samples prepared in buffer, the ones prepared in the biological matrix also exhibited a linear dependence between analyte concentration and the corresponding anodic stripping current intensity. Calibration curves (made in triplicate) (Table 1) likewise showed good linear correlation coefficients.

The relative standard deviation (RSD) associated with the calibration curves slopes for samples prepared in Tris–HNO_3 buffer and diluted urine was, respectively, 13.5% and 11.2% (Table 1). Although high, these values are consistent with the ones obtained for some immunosensors [19]. The immunosensor mode of preparation (direct adsorption of the capture antibody in the working electrode) may also have affected the immunoassay observed results. By physical adsorption, antibodies are randomly adsorbed onto the working electrode surface, some antigen-binding regions being blocked and the biosensor performance reduced [20].

In the analytical field, the ability to obtain the lowest detection limit (LOD) is the most critical feature. The first stage in LOD calculation was the determination of a linear relation between gB concentration and current signal as shown in Fig. 6b and Table 1. The incorrect adjustments due to the existence of anomalous points were avoided using least median squares regression (LMS) [15,16]. Then, in order to check the technique LOD value, results from three calibration curves were evaluated using the DETARCHI program [17], a program that calculates detection limits with an evaluation of the probability of false positive (α) and negative (β), according to ISO11843-2, 2000 [18]. An average limit of detection of 3.3 ± 1.7 ng/mL for HCMV gB in buffer and 3.2 ± 0.2 ng/mL for HCMV gB in urine samples was obtained for $\alpha = \beta = 0.05$ (Table 1).

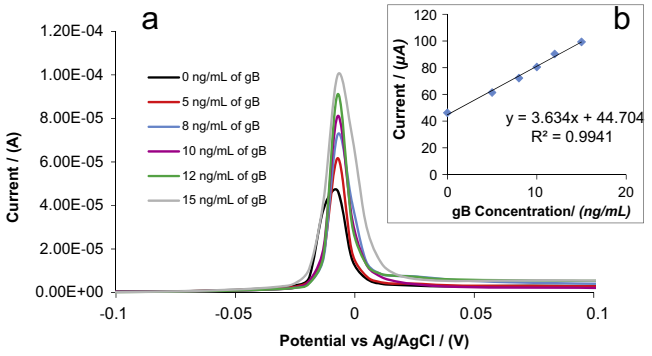

Fig. 6. Linear-sweep stripping voltammetric curves of AgNPs (a) and calibration curve (b) for gB detection using the proposed strategy. gB was diluted in Tris–HNO_3 buffer. (a) Curves are the differential pulse voltammetry responses for gB at concentrations from 0 to 15.0 ng/mL.

Table 1
Calibration parameters for gB prepared in buffer and urine samples (1:2 dilution). RSD—relative standard deviation of analyte detection on different electrodes—i.e. it shows reproducibility of construction of independent biosensor devices.

Matrix	Equation	Slope	R^2	RSD (%)	Detection limit (ng/mL)
Tris–HNO_3 buffer	$y=4.7x+28.0$	4.7	0.9805	13.5	3.3 ± 1.7
	$y=4.6x+65.1$	4.6	0.9906		
	$y=3.6x+44.7$	3.6	0.9941		
Urine	$y=0.7x+8.0$	0.7	0.9945	11.2	3.2 ± 0.2
	$y=0.9x+17.8$	0.9	0.9913		
	$y=0.8x+13.9$	0.8	0.9909		

These two values are similar, leading us to consider that the matrix does not interfere with the immunosensor response.

Finally our results were compared with the ones obtained by Susmel et al. [21]. In their studies a piezoelectric immunosensor for cytomegalovirus gB detection was developed. The immunosensor provided a detection limit of 1 μg/mL and offered a linear range from 2.5 to 5 μg/mL. Comparatively, the present electrochemical immunosensor offers a lower detection limit, being able to detect trace concentrations.

3.3. Application in the analysis of spiked human urine samples

In order to evaluate the analytical reliability of the proposed immunosensor for real sample analysis, the immunosensor was used for the determination of gB in urine samples. Three urine samples collected from healthy humans were spiked with respectively 4, 6 and

Table 2
Results of HCMV glycoprotein B determination in urine samples by application of the standard addition method (*N* is the number of experimental data points).

Concentration of gB in spiked urine sample (ng/mL)	*N*	Standard addition method equation	R^2	Detected content (ng/mL)	Relative error (%)
4.00	5	$y=0.673x+2.91$	0.9914	4.32 ± 0.62	8.1
6.00	4	$y=0.730x+3.87$	0.9924	5.30 ± 0.71	−11.7
7.00	4	$y=0.781x+6.25$	0.9931	8.00 ± 0.78	14.3

7 ng/mL of gB. By application of the standard addition method, the initial concentrations of gB added to each urine sample were obtained (Table 2). A value of 4.32 ± 0.62 ng/mL was found for the lowest gB concentration (relative error 8.1%), a value of 5.30 ± 0.71 ng/mL was found for the sample spiked with 6 ng/mL of gB (relative error −11.7%) and a value of 8.00 ± 0.78 ng/mL was found for the highest added gB concentration (relative error 14.3%). The observed relative error values seem to be in accordance with the ones usually presented for these types of immunosensors [12,19]. For HCMV detection, no standard method is available to compare with this electrochemical immunosensor, since it uses the gB detection and other currently used techniques are based on DNA amplification, antibody screening or virus isolation in fibroblast culture (the gold standard method for HCMV detection) [3]. Nevertheless, the overall analysis time of the proposed biosensor is considerably shorter when compared to the usually applied methods: shell-vial (~24 h), polymerase chain reaction (~6 h) and ELISA assay (at least 4 h). The constructed biosensor can analyze a sample within 50–60 min with 20 min needed for gB incubation plus 20 min more for AuNP-labeled anti-cytomegalovirus glycoprotein B incubation, about 5 min for the anodic stripping voltammetry and a couple of minutes for the electrode washing and the electrode integration within the measurement set-up. In terms of selectivity, as in the case of ELISA tests, the presented immunosensor also presents the possibility of false positive results, caused by cross-reactions with some virus of *Herpesviridae* family, rheumatoid factors and antinuclear antibodies [22].

4. Conclusions

Established in screen-printed electrodes and with the help of nanoparticles, an inexpensive, simple and disposable electrochemical sandwich type immunosensor for glycoprotein B detection in urine is proposed. The basic principle is that a higher concentration of HCMV glycoprotein B means a higher amount of captured gold nanoparticles on the sensor surface, resulting in higher silver deposition with higher measured signals. This study achieved variations in the stripping-current response that directly correlated with the variation in HCMV glycoprotein B concentration. Detection conditions have been optimized. A saturation profile was reached for 15 ng/mL of HCMV gB. The calculated detection limit in urine was 3.2 ± 0.2 ng/mL of HCMV gB, which greatly improves the one obtained by Susmel et al. [21] in the detection of the same protein using a piezoelectric biosensor. The reproducibility, evaluated by the residual standard deviation (RSD), obtained from calibration curves of different assays, was considered acceptable for this type of biosensor (11.2%) [19]. Application of the biosensor in the analysis of spiked urine human samples provided results with relative errors less than 14.3%, indicating that the proposed method accuracy must be improved for detection of HCMV gB in clinical samples. Nevertheless, the possibility of using an electrochemical based immunosensor for the rapid detection of gB in human urine samples (~1 h) was demonstrated. These promising results provide excellent guidelines for device improvement. Affecting the reproducibility of the immunoassay is a deficiency on the antibodies immobilization. As mentioned, by physical adsorption, antibodies are randomly adsorbed onto the working electrode. Ongoing studies are trying different antibody immobilization strategies to improve immunosensor performance.

Acknowledgments

The authors would like to acknowledge funding via Portuguese Foundation for Science and Technology (FCT) for financial support (Strategic project PEst-OE/SAU/UI0709/2014) and via the Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (FEDER) (INNPACTO SERIBIO), (TEC2009-12029) and TEC2013-40561-P as well as through Junta de Castilla y León (BU212A12-2).

References

[1] M.G. Revello, G. Gerna, Clin. Microbiol. Rev. 15 (2002) 680–715.
[2] F. Domingues, F. Sousa, J. Queiroz, Bol. Biotecnol. 82 (2005) 28–35.
[3] B. Yen-lieberman, Clin. Microbiol. Newsl. 22 (2000) 105–109.
[4] S. Ferreira, J. Queiroz, F. Domingues, J. Microbiol. Biotechnol. 16 (2006) 1026–1031.
[5] O.D. Renedo, M.A. Alonso-Lomillo, M.J.A. Martínez, Talanta 73 (2007) 202–219.
[6] S. Zhang, G. Wright, Y. Yang, Biosens. Bioelectron. 15 (2000) 273–282.
[7] A.L. Ghindilis, P. Atanasov, M. Wilkins, E. Wilkins, Biosens. Bioelectron. 13 (1998) 113–131.
[8] A. Warsinke, A. Benkert, F.W. Scheller, J. Anal. Chem. 366 (2000) 622–634.
[9] N.M. White, J.D. Turner, Meas. Sci. Technol. 8 (1997) 1–20.
[10] A. Speckner, D. Glykofrydes, M. Ohlin, M. Mach, J. Gen. Virol. 80 (1999) 2183–2191.
[11] A. Muñiz, C. Parolo, A. Merkoçi, Mater. Today 13 (2010) 24–34.
[12] G. Lai, L. Wang, J. Wu, H. Ju, F. Yan, Anal. Chim. Acta 721 (2012) 1–6.
[13] G. Lai, F. Yan, H. Ju, Anal. Chem. 81 (2009) 9730–9736.
[14] S. Bi, Y. Yan, X. Yang, S. Zhang, Chem. Eur. J. 15 (2009) 4704–4709.
[15] D.L. Massart, L. Kaufman, Anal. Chim. Acta 187 (1986) 171–179.
[16] P.J. Rousseeuw, A.M. Leroy, J. Appl. Econ. 4 (1989) 309–311.
[17] L. Sarabia, M.C. Ortiz, Trends Anal. Chem. 13 (1994) 1–6.
[18] ISO 11843-2: 2000 Capability of detection, 2000.
[19] T. Bertok, A. Sediva, J. Katrlik, P. Gemeiner, M. Mikula, M. Nosko, J. Tkac, Talanta 108 (2013) 11–18.
[20] A.K. Trilling, J. Beekwildera, H. Zuilhof, Analyst 138 (2013) 1619–1627.
[21] S. Susmel, C.K. O'Sullivan, G.G. Guilbault, Enzyme Microb. Technol. 27 (2000) 639–645.
[22] A. Daiminger, U. Bäder, M. Eggers, T. Lazzarotto, G. Enders, J. Clin. Virol. 13 (1999) 161–171.